

## FIGURE 1

cDNA sequence of wild type amFP486

ATGGCTCTTTCAAACAAGTTTATCGGAGATGACATGAAAATGACCTACCATATGGATG  
GCTGTGTCAATGGGCATTACTTTACCGTCAAAGGTGAAGGCAACGGGAAGCCATACGA  
AGGGACGCAGACCTCGACTTTTAAAGTCACCATGGCCAACGGTGGGCCCCCTTGCATT  
TCCTTTGACATACTATCTACAGTGTTCAGTATGGAAATCGATGCTTTACTGCGTATC  
CTACCAGTATGCCCCGACTATTTCAAACAAGCATTTCCTGACGGAATGTCATATGAAAG  
GACTTTTACCTATGAAGATGGAGGAGTTGCTACAGCCAGTTGGGAAATAAGCCTTAAA  
GGCAACTGCTTTGAGCACAAATCCACGTTTCATGGAGTGAACTTTCCTGCTGATGGAC  
CTGTGATGGCGAAGATGACAACCTGGTTGGGACCCATCTTTTGAGAAAATGACTGTCTG  
CGATGGAATATTGAAGGGTGATGTACCCGCGTTCCTCATGCTGCAAGGAGGTGGCAAT  
TACAGATGCCAATTCCACACTTCTTACAAGACAAAAAACCAGGTGACGATGCCACCAA  
ACCATGCGGTGGAACATCGCATTGCGAGGACCGACCTTGACAAAGGTGGCAACAGTGT  
TCAGCTGACGGAGCACGCTGTTGCACATATAACCTCTGTTGTCCCTTTC (SEQ ID  
NO: 01)

amino acid sequence of wild type amFP486

MALSNKFIGD DMKMTYHMDG CVNGHYFTVK GEGNGKPYEG TQTSTFKVTM ANGGPLAFSF  
DILSTVFKYG NRCFTAYPTS MPDYFKQAFP DGMSYERTFT YEDGGVATAS WEISLKGNCF  
EHKSTFHGVN FPADGPVMAK MTTGWDPSE KMTVCDGILK GDVTAFLMLQ GGGNYRCQFH  
TSYKTKKPVT MPPNHAVEHR IARTDLKGG NSVQLTEHAV AHITSVVPF  
(SEQ ID NO: 02)

Figure 2

cDNA sequence of zFP506

ATGGCTCAGTCAAAGCACGGTCTAACAAAAGAAATGACAATGAAATACCGTATGGAAGGGTGC  
GTCGATGGACATAAAATTTGTGATCACGGGAGAGGGCATTTGGATATCCGTTCAAAGGGAAACAG  
GCTATTAATCTGTGTGTGGTCTGAAGGTGGACCATTGCCATTTGCCGAAGACATATTGTCAGCT  
GCCTTTATGTACGGAAACAGGGTTTTCACTGAATATCCTCAAGACATAGCTGACTATTTCAAG  
AACTCGTGTCTGCTGGTTATACATGGGACAGGTCTTTTCTCTTTGAGGATGGAGCAGTTTGC  
ATATGTAATGCAGATATAACAGTGAGTGTGGAAGAAAAGTGCATGTATCATGAGTCCAAATTT  
TATGGAGTGAATTTTCTGCTGATGGACCTGTGATGAAAAGATGACAGATAACTGGGAGCCA  
TCCTGCGAGAAGATCATAACAGTACCTAAGCAGGGGATATTGAAAGGGGATGTCTCCATGTAC  
CTCCTTCTGAAGGATGGTGGGCGTTTACGGTGCCAATTCGACACAGTTTACAAAGCAAAGTCT  
GTGCCAAGAAAGATGCCGGACTGGCACTTCATCCAGCATAAGCTCACCCGTGAAGACCGCAGC  
GATGCTAAGAATCAGAAATGGCATCTGACAGAACATGCTATTGCATCCGGATCTGCATTGCCC  
(SEQ ID NO:03)

amino acid sequence of zFP506

MAQSKHGLTK EMTMKYRMEG CVDGHKFVIT GEGIGYPFKG KQAINLCVVE GGPLPFAEDI LSAAFNYGNR VFTEYPQDIA  
DYFKNSCPAG YTWDRSFLFE DGAVCICNAD ITVSVEENCM YHESKFYGVN FPDGPMVMKK MTDNWEPSCE KIIPVPKQGI  
LKGDVSMYLL LKDGGRRLRCQ FDTVYKAKSV PRKMPDWHFI QHKLTREDRS DAKNQKWHLT EHAIASGSAL P  
(SEQ ID NO:04)

Figure 3

cDNA sequence of zFP538

gagttgagtt tctcgacttc agttgtatca attttggggc atcaagcgat ctattttcaa  
catggctcat tcaaagcacg gtctaaaaga agaaatgaca atgaaatacc acatggaagg  
gtgcgtcaac ggacataaat ttgtgatcac gggcgaaggc attggatata cgttcaaagg  
gaaacagact attaattctgt gtgtgatcga agggggacca ttgccatttt ccgaagacat  
attgtcagct ggctttaagt acggagacag gatttttact gaatatcctc aagacatagt  
agactatttc aagaactcgt gtcctgctgg atatacatgg ggcagggtctt ttctctttga  
ggatggagca gtctgcatat gcaatgtaga tataacagtg agtgtcaaag aaaactgcat  
ttatcataag agcatattta atggaatgaa ttttctgct gatggacctg tgatgaaaaa  
gatgacaact aactgggaag catcctgcca gaagatcatg ccagtaccta agcaggggat  
actgaaaggg gatgtctcca tgtacctcct tctgaaggat ggtgggctgt accggtgccca  
gttcgacaca gtttaciaaag caaagtctgt gccaaagtaag atgccggagt ggcacttcat  
ccagcataag ctctcccgctg aagaccgcag cgatgctaag aatcagaagt ggcagctgac  
agagcatgct attgcattcc cttctgcctt ggccctgataa gaatgtagtt ccaacatttt  
aatgcatgtg cttgtcaatt attctgataa aaatgtagtt gagttgaaaa cagacaagta  
caaataaagc acatgtaa at cgtct (SEQ ID NO:05)

amino acid sequence of zFP538

Met Ala His Ser Lys His Gly Leu Lys Glu Glu Met Thr Met Lys  
Tyr His Met Glu Gly Cys Val Asn Gly His Lys Phe Val Ile Thr  
Gly Glu Gly Ile Gly Tyr Pro Phe Lys Gly Lys Gln Thr Ile Asn  
Leu Cys Val Ile Glu Gly Gly Pro Leu Pro Phe Ser Glu Asp Ile  
Leu Ser Ala Gly Phe Lys Tyr Gly Asp Arg Ile Phe Thr Glu Tyr  
Pro Gln Asp Ile Val Asp Tyr Phe Lys Asn Ser Cys Pro Ala Gly  
Tyr Thr Trp Gly Ser Phe Leu Phe Glu Asp Gly Ala Val Cys Ile  
Cys Asn Val Asp Ile Thr Val Ser Val Lys Glu Asn Cys Ile Tyr  
His Lys Ser Ile Phe Asn Gly Met Asn Phe Pro Ala Asp Gly Pro  
Val Met Lys Lys Met Thr Thr Asn Trp Glu Ala Ser Cys Glu Lys  
Ile Met Pro Val Pro Lys Gln Gly Ile Leu Lys Gly Asp Val Ser  
Met Tyr Leu Leu Leu Lys Asp Gly Arg Tyr Arg Cys Gln Phe  
Asp Thr Val Tyr Lys Ala Lys Ser Val Pro Ser Lys Met Pro Glu  
Trp His Phe Ile Gln His Lys Leu Leu Arg Glu Asp Arg Ser Asp  
Ala Lys Asn Gln Lys Trp Gln Leu Thr Glu His Ala Ile Ala Phe  
Pro Ser Ala Leu Ala (SEQ ID NO:06)

## FIGURE 4

All mutants are derived from drFP583 (called "pink" or FP6.) by random mutagenesis

The mutants E57 and AG4 are derivative from E5

Mutant: E5 = V105A, S197T Phenotype: in *E. coli* seen as Green overnight, matures to Red over 24h at 37°C (final peaks ratio Red vs. Green is 75:25); folding is faster then FP6.

Mutant: E8 = N42H Phenotype: always has two peaks Green & Red in approx. 60:40; folding is faster than E5 (about 8h at 37°C)

Mutant: E83 = N42H, V71A, I180V Phenotype: always has two almost equal peaks Green & Red; folding is the same as for E8

Mutant: E5up = V105A Phenotype: seen as Red from the beginning; folding is faster than E5 (about 12-16h) Almost no Green peak at final point of maturation

Mutant: E57 = V105A, I161T, S197A Phenotype: at common is like E5up but folding is more faster (no more that 8-10h) Very small Green peak at final point of maturation (less that 5%)

Mutant: E5down = S197T Phenotype and folding rate are exactly the same as for E5

Mutant: AG4 = V71M, V105A, S197T Phenotype: Very bright Green, no Red at all (even at the beginning); folding is faster than E5 (no more that 16h)

Mutant: AG4 = V71M, V105A, Y120H, S197T Phenotype: at common is like AG4, but more brighter (approx. twice) one.

1	Met	Arg	Ser	Ser	Lys	Asn	Val	Ile	Lys	Glu	Phe	Met	Arg	Phe	Lys	Val	16
1	ATG	CGC	TCC	TCC	AAG	AAC	GTC	ATC	AAG	GAG	TTC	ATG	CGC	TTC	AAG	GTG	48
17	Arg	Met	Glu	Gly	Thr	Val	Asn	Gly	His	Glu	Phe	Glu	Ile	Glu	Gly	Glu	32
49	CGC	ATG	GAG	GGC	ACC	GTG	AAC	GGC	CAC	GAG	TTC	GAG	ATC	GAG	GGC	GAG	96
His(CAC) for E8 and E83																	
33	Gly	Glu	Gly	Arg	Pro	Tyr	Glu	Gly	His	Asn	Thr	Val	Lys	Leu	Lys	Val	48
97	GGC	GAG	GGC	CGC	CCC	TAC	GAG	GGC	CAC	AAC	ACC	GTG	AAG	CTG	AAG	GTG	144
49	Thr	Lys	Gly	Gly	Pro	Leu	Pro	Phe	Ala	Trp	Asp	Ile	Leu	Ser	Pro	Gln	64
145	ACC	AAG	GGC	GGC	CCC	CTG	CCC	TTC	GCC	TGG	GAC	ATC	CTG	TCC	CCC	CAG	192
Met(ATG) for AG4 and AG45/Ala(GCG) for E83																	
65	Phe	Gln	Tyr	Gly	Ser	Lys	Val	Tyr	Val	Lys	His	Pro	Ala	Asp	Ile	Pro	80
193	TTC	CAG	TAC	GGC	TCC	AAG	GTG	TAC	GTG	AAG	CAC	CCC	GCC	GAC	ATC	CCC	240
81	Asp	Tyr	Lys	Lys	Leu	Ser	Phe	Pro	Glu	Gly	Phe	Lys	Trp	Glu	Arg	Val	96
241	GAC	TAC	AAG	AAG	CTG	TCC	TTC	CCC	GAG	GGC	TTC	AAG	TGG	GAG	CGC	GTG	288
Ala(GCG) -for E5, E57, AG4 and AG45																	
97	Met	Asn	Phe	Glu	Asp	Gly	Gly	Val	Val	Thr	Val	Thr	Gln	Asp	Ser	Ser	112
289	ATG	AAC	TTC	GAG	GAC	GGC	GGC	GTG	GTG	ACC	GTG	ACC	CAG	GAC	TCC	TCC	336
His(CAC) -for AG45																	
113	Leu	Gln	Asp	Gly	Cys	Phe	Ile	Tyr	Lys	Val	Lys	Phe	Ile	Gly	Val	Asn	128
337	CTG	CAG	GAC	GGC	TGC	TTC	ATC	TAC	AAG	GTG	AAG	TTC	ATC	GGC	GTG	AAC	384
129	Phe	Pro	Ser	Asp	Gly	Pro	Val	Met	Gln	Lys	Lys	Thr	Met	Gly	Trp	Glu	144
385	TTC	CCC	TCC	GAC	GGC	CCC	GTG	ATG	CAG	AAG	AAG	ACC	ATG	GGC	TGG	GAG	432
145	Ala	Ser	Thr	Glu	Arg	Leu	Tyr	Pro	Arg	Asp	Gly	Val	Leu	Lys	Gly	Glu	160
433	GCC	TCC	ACC	GAG	CGC	CTG	TAC	CCC	CGC	GAC	GGC	GTG	CTG	AAG	GGC	GAG	480
Thr(ACC) for E57																	
161	Ile	His	Lys	Ala	Leu	Lys	Leu	Lys	Asp	Gly	Gly	His	Tyr	Leu	Val	Glu	176
481	ATC	CAC	AAG	GCC	CTG	AAG	CTG	AAG	GAC	GGC	GGC	CAC	TAC	CTG	GTG	GAG	528
Val(GTC) for E83																	
177	Phe	Lys	Ser	Ile	Tyr	Met	Ala	Lys	Lys	Pro	Val	Gln	Leu	Pro	Gly	Tyr	192
529	TTC	AAG	TCC	ATC	TAC	ATG	GCC	AAG	AAG	CCC	GTG	CAG	CTG	CCC	GGC	TAC	576
Thr(ACC) for E5, AG4 and AG45/Ala(GCC) for E57																	
193	Tyr	Tyr	Val	Asp	Ser	Lys	Leu	Asp	Ile	Thr	Ser	His	Asn	Glu	Asp	Tyr	208
577	TAC	TAC	GTG	GAC	TCC	AAG	CTG	GAC	ATC	ACC	TCC	CAC	AAC	GAG	GAC	TAC	624
209	Thr	Ile	Val	Glu	Gln	Tyr	Glu	Arg	Thr	Glu	Gly	Arg	His	His	Leu	Phe	Leu *** 229
625	ACC	ATC	GTG	GAG	CAG	TAC	GAG	CGC	ACC	GAG	GGC	CGC	CAC	CAC	CTG	TTC	CTG TAA 678

(SEQ ID NO:07 & 8)

## FIGURE 5

### Amino Acid and Nucleotide Sequence for asFP600

ATGGCTTCCTTTTAAAGAAGACTATGCCCTTTAAGACGACCATTGAAGGGACGGTTAATGGCCAC  
TACTTCAAGTGTACAGGAAAAGGAGAGGGCAACCCATTTGAGGGTACGCAGGAAATGAAGATAGAG  
GTCATCGAAGGAGGTCCATTGCCATTTGCCTTCCACATTTTGTCAACGAGTTGTATGTACGGTAGT  
AAGGCCTTCATCAAGTATGTGTCAGGAATTCTGACTACTTCAAGCAGTCTTCCCTGAAGGTTTT  
ACTTGGGAAAGAACCACAACCTACGAGGATGGAGGCTTCTTACAGCTCATCAGGACACAAGCCTA  
GATGGAGATTGCCTCGTTTACAAGGTCAAGATTCTTGGTAATAATTTTCTGCTGATGGCCCCGTG  
ATGCAGAACAAAGCAGGAAGATGGGAGCCATCCACCGAGATAGTTTATGAAGTTGACGGTGTCTG  
CGTGGACAGTCTTTGATGGCCCTTAAGTGCCCTGGTGGTTCGTCATCTGACTTGCCATCTCCATACT  
ACTTACAGGTCCAAAAAACCAGCTGCTGCCTTGAAGATGCCAGGATTTTCATTTTGAAGATCATCGC  
ATCGAGATAATGGAGGAAGTTGAGAAAGGCAAGTGCTATAAACAGTACGAAGCAGCAGTGGGCAGG  
TACTGTGATGCTGCTCCATCCAAGCTTGGACATAAC (SEQ ID NO:09)

### Amino acid

MASFLKKTMP FKTTIEGTVN GHYFKCTGKG EGNPFEGTQE MKIEVIEGGP LPFAFHILST  
SCMYGSKTFI KYVSGIPDYF KQSFPEGFTW ERTTTYEDGG FLTAHQDTSL DGDCLVYKVK  
ILGNNFPADG PVMQNKAGRW EPATEIVYEV DGVLRGQSLM ALKCPGGRHL TCHLHTTYRS  
KKPAAALKMP GFHFEDHRIE IMEEVEKGKC YKQYEA AVGR YCDAAPSKLG HN (SEQ ID  
NO:10)

Figure 6

Sequence of humanized 6/9 hybrid gene and 6/9-Q3 mutant																	
for 6/9-2G and 6/9-Q3 CAG(Q)																	
1	ATG	AGC	TGC	AGC	AAG	AAC	GTG	ATC	AAG	GAG	TTC	ATG	CGG	TTC	<b>AAG</b>	GTG	48
1	M	S	C	S	K	N	V	I	K	E	F	M	R	F	<u>K</u>	V	16
49	CGG	ATG	GAG	GGC	ACC	GTG	AAC	GGC	CAC	GAG	TTC	GAG	ATC	AAG	GGC	GAG	96
17	R	M	E	G	T	V	N	G	H	E	F	E	I	K	G	E	32
97	GGC	GAG	GGC	CGG	CCC	TAC	GAG	GGC	CAC	TGC	AGC	GTG	AAG	CTC	ATG	GTG	144
33	G	E	G	R	P	Y	E	G	H	C	S	V	K	L	M	V	48
145	ACC	AAG	GGC	GGC	CCC	CTC	CCC	TTC	GCC	TTC	GAC	ATC	CTC	AGC	CCC	CAG	192
49	T	K	G	G	P	L	P	F	A	F	D	I	L	S	P	Q	64
193	TTC	CAG	TAC	GGC	AGC	AAG	GTG	TAC	GTG	AAG	CAC	CCC	GCC	GAC	ATC	CCC	240
65	F	Q	Y	G	S	K	V	Y	V	K	H	P	A	D	I	P	80
ATG(M) for 6/9-Q3																	
241	GAC	TAC	<b>AAG</b>	AAG	CTC	AGC	TTC	CCC	GAG	GGC	TTC	AAG	TGG	GAG	CGG	GTG	288
81	D	Y	<u>K</u>	K	L	S	F	P	E	G	F	K	W	E	R	V	96
289	ATG	AAC	TTC	GAG	GAC	GGC	GGC	GTG	GTG	ACC	GTG	AGC	CAG	GAC	AGC	AGC	336
97	M	N	F	E	D	G	G	V	V	T	V	S	Q	D	S	S	112
337	CTC	AAG	GAC	GGC	TGC	TTC	ATC	TAC	GAG	GTG	AAG	TTC	ATC	GGC	GTG	AAC	384
113	L	K	D	G	C	F	I	Y	E	V	K	F	I	G	V	N	128
385	TTC	CCC	AGC	GAC	GGC	CCC	GTG	ATG	CAG	CGG	CGG	ACC	CGG	GGC	TGG	GAG	432
129	F	P	S	D	G	P	V	M	Q	R	R	T	R	G	W	E	144
433	GCC	AGC	AGC	GAG	CGG	CTC	TAC	CCC	CGG	GAC	GGC	GTG	CTC	AAG	GGC	GAC	480
145	A	S	S	E	R	L	Y	P	R	D	G	V	L	K	G	D	160
481	ATC	CAC	ATG	GCC	CTC	CGG	CTC	GAG	GGC	GGC	GGC	CAC	TAC	CTC	GTG	GAG	528
161	I	H	M	A	L	R	L	E	G	G	G	H	Y	L	V	E	176
529	TTC	AAG	AGC	ATC	TAC	ATG	GCC	AAG	AAG	CCC	GTG	CAG	CTC	CCC	GGC	TAC	576
177	F	K	S	I	Y	M	A	K	K	P	V	Q	L	P	G	Y	192
577	TAC	TAC	GTG	GAC	AGC	AAG	CTC	GAC	ATC	ACC	AGC	CAC	AAC	GAG	GAC	TAC	624
193	Y	Y	V	D	S	K	L	D	I	T	S	H	N	E	D	Y	208
TCC(S) for 6/9-2G and 6/9-Q3																	
625	ACC	ATC	GTG	GAG	CAG	TAC	GAG	CGG	<b>ACC</b>	GAG	GGC	CGG	CAC	CAC	CTC	TTC	672
209	T	I	V	E	Q	Y	E	R	<u>T</u>	E	G	R	H	H	L	F	224
673	CTC	TGA															678
225	L	*															226

(SEQ ID NO:11 & 12)

Figure 7

Nucleic acid sequence FP6 (E57)-NA

ATGGCCTCCTCCGAGAACGTCATCACCGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCACCGTGA  
ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCCACAACACCGTG  
AAGCTGAAGGTGACCAAGGGCGGCCCTGCCCTTCGCTGGGACATCCTGTCCCCCAGTTCCAGT  
ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCCTTCCCCGA  
GGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGCGGACCGTGACCCAGGACTC  
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATCGGCGTGAACCTCCCCCTCCGACGGC  
CCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGC  
GTGCTGAAGGGCGAGACCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTC  
AAGTCCATCTACATGGCCAAGAAGCCCGTGACGCTGCCCGGCTACTACTACGTGGACGCCAAGCTGG  
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCCGCCACCA  
CCTGTTCTTG (SEQ ID NO:13)

Figure 8

DNA sequence (ORF) of E5-NA

ATGGCCTCCTCCGAGAACGTCATCACCGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCACCGTGA  
ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCCACAACACCGTG  
AAGTTGAAGGTGACCAAGGGCGGCCCTGCCCTTCGCTGGGACATCCTGTCCCCCAGTTCCAGT  
ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCCTTCCCCGA  
GGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGCGGACCGTGACCCAGGACTC  
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATCGGCGTGAACCTCCCCCTCCGACGGC  
CCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGC  
GTGCTGAAGGGCGAGATCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTC  
AAGTCCATCTACATGGCCAAGAAGCCCGTGACGCTGCCCGGCTACTACTACGTGGACACCAAGCTGG  
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCCGCCACCA  
CCTGTTCTTGTA (SEQ ID NO:14)

Figure 9

Non-aggregating mutant FP3-NA was generated from zFP506-N65M (non-humanized version). In comparison with zFP506-N65M, FP3-NA contains two additional amino acid substitutions - K5E and K10E. Also, one accidental nucleotide substitution was introduced due to PCR mistake (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```

GGA TCC GCT CAG TCA GAG CAC GGT CTA ACA GAA GAA ATG ACA ATG AAA
BamHI  A  Q  S  E  H  G  L  T  E  E  M  T  M  K

TAC CGT ATG GAA GGG TGC GTC GAT GGA CAT AAA TTT GTG ATC ACG GGA
Y  R  M  E  G  C  V  D  G  H  K  F  V  I  T  G

GAG GGC ATT GGA TAT CCG TTC AAA GGG AAA CAG GCT ATT AAT CTG TGT
E  G  I  G  Y  P  F  K  G  K  Q  A  I  N  L  C

GTG GTC GAA GGT GGA CCA TTG CCA TTT GCC GAA GAC ATA TTG TCA GCT
V  V  E  G  G  P  L  P  F  A  E  D  I  L  S  A

GCC TTT ATG TAC GGA AAC AGG GTT TTC ACT GAA TAT CCT CAA GAC ATA
A  F  M  Y  G  N  R  V  F  T  E  Y  P  Q  D  I

GTT GAC TAT TTC AAG AAC TCG TGT CCT GCT GGA TAT ACA TGG GAC AGG
V  D  Y  F  K  N  S  C  P  A  G  Y  T  W  D  R

TCT TTT CTC TTT GAG GAT GGA GCA GTT TGC ATA TGT AAT GCA GAT ATA
S  F  L  F  E  D  G  A  V  C  I  C  N  A  D  I

ACA GTG AGT GTT GAA GAA AAC TGC ATG TAT CAT GAG TCC AAA TTC TAT
T  V  S  V  E  E  N  C  M  Y  H  E  S  K  F  Y

GGA GTG AAT TTT CCT GCT GAT GGA CCT GTG ATG AAA AAG ATG ACA GAT
G  V  N  F  P  A  D  G  P  V  M  K  K  M  T  D

AAC TGG GAG CCA TCC TGC GAG AAG ATC ATA CCA GTA CCT AAG CAG GGG
N  W  E  P  S  C  E  K  I  I  P  V  P  K  Q  G

ATA TTG AAA GGG GAT GTC TCC ATG TAC CTC CTT CTG AAG GAT GGT GGG
I  L  K  G  D  V  S  M  Y  L  L  L  K  D  G  G

CGT TTA CGG TGC CAA TTC GAC ACA GTT TAC AAA GCA AAG TCT GTG CCA
R  L  R  C  Q  F  D  T  V  Y  K  A  K  S  V  P

AGA AAG ATG CCG GAC TGG CAC TTC ATC CAG CAT AAG CTC ACC CGT GAA
R  K  M  P  D  W  H  F  I  Q  H  K  L  T  R  E

GAC CGC AGC GAT GCT AAG AAT CAG AAA TGG CAT CTG ACA GAA CAT GCT
D  R  S  D  A  K  N  Q  K  W  H  L  T  E  H  A

ATT GCA TCC GGA TCT GCA TTG CCC TGA AAGCTT
I  A  S  G  S  A  L  P  *  HindIII  (SEQ ID NO:15 & 16)

```

Figure 10

Non-aggregating mutant FP4-NA was generated from zFP538-M128V (humanized version). In comparison with zFP538-M128V, FP4-NA contains two additional amino acid substitutions - K5E and K9T. Also, two accidental nucleotide substitutions were introduced due to PCR mistakes (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```

GGA TCC GCC CAC AGC GAG CAC GGC CTG ACC GAG GAG ATG ACC ATG AAG
BamHI  A  H  S  E  H  G  L  T  E  E  M  T  M  K

TAC CAC ATG GAG GGC TGC GTG AAC GGC CAC AAG TTC GTG ATC ACC GGC
Y  H  M  E  G  C  V  N  G  H  K  F  V  I  T  G

GAG GGC ATC GGC TAC CCC TTC AAG GGC AAG CAG ACC ATC AAC CTG TGC
E  G  I  G  Y  P  F  K  G  K  Q  T  I  N  L  C

GTG ATC GAG GGC GGC CCC CTG CCC TTC AGC GAG GAC ATC CTG AGC GCC
V  I  E  G  G  P  L  P  F  S  E  D  I  L  S  A

GGC TTC AAG TAC GGC GAC CGG ATC TTC ACC GAG TAC CCC CAG GAC ATC
G  F  K  Y  G  D  R  I  F  T  E  Y  P  Q  D  I

GTG GAC TAC TTC AAG AAC AGC TGC CCC GCC GGC TAC ACC TGG GGC CGG
V  D  Y  F  K  N  S  C  P  A  G  Y  T  W  G  R

AGC TTC CTG TTC GAG GAC GGC GCC GTG TGC ATC TGT AAC GTG GAC ATC
S  F  L  F  E  D  G  A  V  C  I  C  N  V  D  I

ACC GTG AGC GTG AAG GAG AAC TGC ATC TAC CAC AAG AGC ATC TTC AAC
T  V  S  V  K  E  N  C  I  Y  H  K  S  I  F  N

GGC GTG AAC TTC CCC GCC GAC GGC CCC GTG ATG AAG AAG ATG ACC ACC
G  V  N  F  P  A  D  G  P  V  M  K  K  M  T  T

AAC TGG GAG GCC AGC TGC GAG AAG ATC ATG CCC GTG CCT AAC CAG GGC
N  W  E  A  S  C  E  K  I  M  P  V  P  K  Q  G

ATC CTG AAG GGC GAC GTG AGC ATG TAC CTG CTG CTG AAG GAC GGC GGC
I  L  K  G  D  V  S  M  Y  L  L  L  K  D  G  G

CGG TAC CGG TGC CAG TTC GAC ACC GTG TAC AAG GCC AAG AGC GTG CCC
R  Y  R  C  Q  F  D  T  V  Y  K  A  K  S  V  P

AGC AAG ATG CCC GAG TGG CAC TTC ATC CAG CAC AAG CTG CTG CGG GAG
S  K  M  P  E  W  H  F  I  Q  H  K  L  L  R  E

GAC CGG AGC GAC GCC AAG AAC CAG AAG TGG CAG CTG ACC GAG CAC GCC
D  R  S  D  A  K  N  Q  K  W  Q  L  T  E  H  A

ATC GCC TTC CCC AGC GCC CTG GCC TGA AAGCTT
I  A  F  P  S  A  L  A  *  HindIII
  
```

(SEQ ID NOS: 17 & 18)

Figure 11

```

      M   A   L   S   N   E   F   I   G   D   D   M   K   M
676   ATG GCC CTG TCC AAC GAG TTC ATC GGC GAC GAC ATG AAG ATG
      TAC CGG GAC AGG TTG TTC AAG TAG CCG CTG CTG TAC TTC TAC
      T   Y   H   M   D   G   C   V   N   G   H   Y   F   T   V
721   ACC TAC CAC ATG GAC GGC TGC GTG AAC GGC CAC TAC TTC ACC GTG
      TGG ATG GTG TAC CTG CCG ACG CAC TTG CCG GTG ATG AAG TGG CAC
      K   G   E   G   S   G   P   Y   E   G   T   Q   T   S
766   AAG GGC GAG GGC AGC GGC AAG CCC TAC GAG GGC ACC CAG ACC TCC
      TTC CCG CTC CCG TCG CCG TTC GGG ATG CTC CCG TGG GTC TGG AGG
      T   F   K   V   T   M   A   N   G   G   P   L   A   F   S
811   ACC TTC AAG GTG ACC ATG GCC AAC GGC GGC CCC CTG GCC TTC TCC
      TGG AAG TTC CAC TGG TAC CGG TTG CCG CCG GGG GAC CGG AAG AGG
      F   D   I   L   S   T   V   F   M   Y   G   N   R   C   F
856   TTC GAC ATC CTG TCC ACC GTG TTC ATG TAC GGC AAC CGC TGC TTC
      AAG CTG TAG GAC AGG TGG CAC AAG TAC ATG CCG TTG GCG ACG AAG
      T   A   Y   P   T   S   M   P   D   Y   F   K   Q   A   F
901   ACC GCC TAC CCC ACC AGC ATG CCC GAC TAC TTC AAG CAG GCC TTC
      TGG CGG ATG GGG TGG TCG TAC GGG CTG ATG AAG TTC GTC CGG AAG
      P   D   G   M   S   Y   E   R   T   F   T   Y   E   D   G
946   CCC GAC GGC ATG TCC TAC GAG AGA ACC TTC ACC TAC GAG GAC GGC
      GGG CTG CCG TAC AGG ATG CTC TCT TGG AAG TGG ATG CTC CTG CCG
      G   V   A   T   A   S   W   E   I   S   L   K   G   N   C
991   GGC GTG GCC ACC GCC AGC TGG GAG ATC AGC CTG AAG GGC AAC TGC
      CCG CAC CGG TGG CGG TCG ACC CTC TAG TCG GAC TTC CCG TTG ACG
      F   E   H   K   S   T   F   H   G   V   N   F   P   A   D
1036  TTC GAG CAC AAG TCC ACC TTC CAC GGC GTG AAC TTC CCC GCC GAC
      AAG CTC GTG TTC AGG TGG AAG GTG CCG CAC TTG AAG GGG CGG CTG
      G   P   V   M   A   K   K   T   T   G   W   D   P   S   F
1081  GGC CCC GTG ATG GCC AAG AAG ACC ACC GGC TGG GAC CCC TCC TTC
      CCG GGG CAC TAC CGG TTC TTC TGG TGG CCG ACC CTG GGG AGG AAG
      E   K   M   T   V   C   D   G   I   L   K   G   D   V   T
1126  GAG AAG ATG ACC GTG TGC GAC GGC ATC TTG AAG GGC GAC GTG ACC
      CTC TTC TAC TGG CAC ACG CTG CCG TAG AAC TTC CCG CTG CAC TGG
      A   F   L   M   L   Q   G   G   G   N   Y   R   C   Q   F
1171  GCC TTC CTG ATG CTG CAG GGC GGC GGC AAC TAC AGA TGC CAG TTC
      CGG AAG GAC TAC GAC GTC CCG CCG CCG TTG ATG TCT ACG GTC AAG
      H   T   S   Y   K   T   K   K   P   V   T   M   P   P   N
1216  CAC ACC TCC TAC AAG ACC AAG AAG CCC GTG ACC ATG CCC CCC AAC
      GTG TGG AGG ATG TTC TGG TTC TTC GGG CAC TGG TAC GGG GGG TTG
      H   V   V   E   H   R   I   A   R   T   D   L   D   K   G
1261  CAC GTG GTG GAG CAC CGC ATC GCC AGA ACC GAC CTG GAC AAG GGC
      GTG CAC CAC CTC GTG GCG TAG CCG TCT TGG CTG GAC CTG TTC CCG
      G   N   S   V   Q   L   T   E   H   A   V   A   H   I   T
1306  GGC AAC AGC GTG CAG CTG ACC GAG CAC GCC GTG GCC CAC ATC ACC
      CCG TTG TCG CAC GTC GAC TGG CTC GTG CGG CAC CGG GTG TAG TGG
      S   V   V   P   F   *
1351  TCC GTG GTG CCC TTC TGA
      AGG CAC CAC GGG AAG ACT

```

(SEQ ID NO:19 & 20)

Figure 12

Non-aggregating mutant FP7-NA was generated from M35-5 (FP7a). In comparison with M35-5, FP7-NA contains two additional substitutions - K6T and K7E. Nucleotide substitutions in the codon for Leu-4 were introduced to optimize codon usage (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```

GGA TCC GCC TCC CTG CTG ACC GAG ACC ATG CCC TTC AGG ACC ACC ATC
BamHI  A  S  L  L  T  E  T  M  P  F  R  T  T  I

GAG GGC ACC GTG AAC GGC CAC TAC TTC AAG TGC ACC GGC AAG GGC GAG
E  G  T  V  N  G  H  Y  F  K  C  T  G  K  G  E

GGC AAC CCC CTC GAG GGC ACC CAG GAG ATG AAG ATC GAG GTG ATC GAG
G  N  P  L  E  G  T  Q  E  M  K  I  E  V  I  E

GGC GGC CCC CTG CCC TTC GCC TTC CAC ATC CTG TCC ACC TCC TGC ATG
G  G  P  L  P  F  A  F  H  I  L  S  T  S  C  M

TAC GGC TCC AAG GCC TTC ATC AAG TAC GTG TCC GGC ATC CCC GAC TAC
Y  G  S  K  A  F  I  K  Y  V  S  G  I  P  D  Y

TTC AAG CAG TCC CTC CCC GAG GGC TTC ACC TGG GAG CGC ACC ACC ACC
F  K  Q  S  L  P  E  G  F  T  W  E  R  T  T  T

TAC GAG GAC GGC GGC TTC CTG ACC GCC CAC CAG GAC ACC TCC CTG GAC
Y  E  D  G  G  F  L  T  A  H  Q  D  T  S  L  D

GGC GAC TGC CTG GTG TAC AAG GTG AAG ATC CTG GGC AAC AAC TTC CCC
G  D  C  L  V  Y  K  V  K  I  L  G  N  N  F  P

GCC GAC GGC CCC GTG ATG CAG AAC AAG GCC GGC CGC TGG GAG CCC TCC
A  D  G  P  V  M  Q  N  K  A  G  R  W  E  P  S

ACC GAG ATC GTG TAC GAG GTG GAC GGC GTG CTG CGC GGC CAG TCC CTG
T  E  I  V  Y  E  V  D  G  V  L  R  G  Q  S  L

ATG GCC CTG GAG TGC CCC GGC GGT CGC CAC CTG ACC TGC CAC CTG CAC
M  A  L  E  C  P  G  G  R  H  L  T  C  H  L  H

ACC ACC TAC CGC TCC AAG AAG CCC GCC TCC GCC CTG AAG ATG CCC GGC
T  T  Y  R  S  K  K  P  A  S  A  L  K  M  P  G

TTC CAC TTC GAG GAC CAC CGC ATC GAG ATC CTG GAG GAG GTG GAG AAG
F  H  F  E  D  H  R  I  E  I  L  E  E  V  E  K

GGC AAG TGC TAC AAG CAG TAC GAG GCC GCC GTG GGC CGC TAC TGC GAC
G  K  C  Y  K  Q  Y  E  A  A  V  G  R  Y  C  D

GCC GCC CCC TCC AAG CTG GGC CAC AAC TGAAGCTT
A  A  P  S  K  L  G  H  N  * HindIII (SEQ ID NO:21 & 22)

```

Figure 13

Dimeric mutant AsRed M35-5D (in comparison to M355NA it carries one amino acid substitution L166S according to GFP numbering; L159S according to self-numbering)

```

      ATG GCC TCC CTG CTG ACC GAG ACC ATG CCC TTC AGG ACC ACC ATC
      M  A  S  L  L  T  E  T  M  P  F  R  T  T  I

GAG GGC ACC GTG AAC GGC CAC TAC TTC AAG TGC ACC GGC AAG GGC GAG
E  G  T  V  N  G  H  Y  F  K  C  T  G  K  G  E

GGC AAC CCC CTC GAG GGC ACC CAG GAG ATG AAG ATC GAG GTG ATC GAG
G  N  P  L  E  G  T  Q  E  M  K  I  E  V  I  E

GGC GGC CCC CTG CCC TTC GCC TTC CAC ATC CTG TCC ACC TCC TGC ATG
G  G  P  L  P  F  A  F  H  I  L  S  T  S  C  M

TAC GGC TCC AAG GCC TTC ATC AAG TAC GTG TCC GGC ATC CCC GAC TAC
Y  G  S  K  A  F  I  K  Y  V  S  G  I  P  D  Y

TTC AAG CAG TCC CTC CCC GAG GGC TTC ACC TGG GAG CGC ACC ACC ACC
F  K  Q  S  L  P  E  G  F  T  W  E  R  T  T  T

TAC GAG GAC GGC GGC TTC CTG ACC GCC CAC CAG GAC ACC TCC CTG GAC
Y  E  D  G  G  F  L  T  A  H  Q  D  T  S  L  D

GGC GAC TGC CTG GTG TAC AAG GTG AAG ATC CTG GGC AAC AAC TTC CCC
G  D  C  L  V  Y  K  V  K  I  L  G  N  N  F  P

GCC GAC GGC CCC GTG ATG CAG AAC AAG GCC GGC CGC TGG GAG CCC TCC
A  D  G  P  V  M  Q  N  K  A  G  R  W  E  P  S

ACC GAG ATC GTG TAC GAG GTG GAC GGC GTG CTG CGC GGC CAG TCC AGC
T  E  I  V  Y  E  V  D  G  V  L  R  G  Q  S  S

ATG GCC CTG GAG TGC CCC GGC GGT CGC CAC CTG ACC TGC CAC CTG CAC
M  A  L  E  C  P  G  G  R  H  L  T  C  H  L  H

ACC ACC TAC CGC TCC AAG AAG CCC GCC TCC GCC CTG AAG ATG CCC GGC
T  T  Y  R  S  K  K  P  A  S  A  L  K  M  P  G

TTC CAC TTC GAG GAC CAC CGC ATC GAG ATC CTG GAG GAG GTG GAG AAG
F  H  F  E  D  H  R  I  E  I  L  E  E  V  E  K


GGC AAG TGC TAC AAG CAG TAC GAG GCC GCC GTG GGC CGC TAC TGC GAC
G  K  C  Y  K  Q  Y  E  A  A  V  G  R  Y  C  D

GCC GCC CCC TCC AAG CTG GGC CAC AAC TGA
A  A  P  S  K  L  G  H  N  *

```

(SEQ ID NO:23 & 24)

Figure 14



MRSSKNVIKEFMRFKVRMEGTVNGHE drFP583  
MSCSKNVIKEFMRFQVRMEGTVNGHE ds/drFP616  
MAQSKHGLTKEMTMKYRMEGCVDGHK zFP506  
MAHSKHGLKEEMTMKYHMEGCVNGHK zFP538  
MALSNKFIGDDMKMTYHMDGCVNGHY amFP486  
MASFLKKTMPFKTTIEGTVNGHY asFP595

(SEQ ID NOS:25-30)

Figure 15

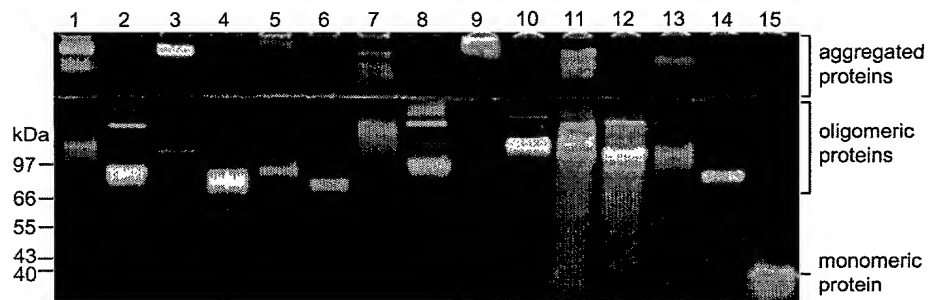


Figure 16

